Novel monoclonal antibodies to study tissue regeneration in planarians.

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Public Summary:
Planarians are an attractive model organism for studying stem cell-based regeneration due to their ability to replace all of their tissues from a population of adult stem cells. The molecular toolkit for planarian research includes the ability to visualize gene expression via whole animal in situ hybridizations. However, there are few antibodies available to determine protein expression. We created monoclonal antibodies that can be used to label muscle fibers, axonal projections in the central and peripheral nervous systems, two populations of intestinal cells, ciliated cells, a subset of stem cell progeny, and discrete cells within the central nervous system as well as the regeneration blastema. We tested these antibodies using eight variations of a formaldehyde-based fixation protocol and determined reliable protocols for immunolabeling whole planarians with each antibody. Our experiments show that some antibodies can be used alongside markers commonly used in planarian research and will be a valuable resource for planarian research.

Scientific Abstract:
BackgroundPlanarians are an attractive model organism for studying stem cell-based regeneration due to their ability to replace all of their tissues from a population of adult stem cells. The molecular toolkit for planarian studies currently includes the ability to study gene function using RNA interference (RNAi) and observe gene expression via in situ hybridizations. However, there are few antibodies available to visualize protein expression, which would greatly enhance analysis of RNAi experiments as well as allow further characterization of planarian cell populations using immunocytochemistry and other immunological techniques. Thus, additional, easy-to-use, and widely available monoclonal antibodies would be advantageous to study regeneration in planarians.ResultsWe have created seven monoclonal antibodies by inoculating mice with formaldehyde-fixed cells isolated from dissociated 3-day regeneration blastemas. These monoclonal antibodies can be used to label muscle fibers, axonal projections in the central and peripheral nervous systems, two populations of intestinal cells, ciliated cells, a subset of neoblast progeny, and discrete cells within the central nervous system as well as the regeneration blastema. We have tested these antibodies using eight variations of a formaldehyde-based fixation protocol and determined reliable protocols for immunolabeling whole planarians with each antibody. We found that labeling efficiency for each antibody varies greatly depending on the addition or removal of tissue processing steps that are commonly used for in situ hybridization or immunolabeling techniques. Our experiments show that a subset of the antibodies can be used alongside markers commonly used in planarian research, including anti-SYNAPSIN and anti-SMEDWI, or following whole-mount in situ hybridization experiments.ConclusionsThe monoclonal antibodies described in this paper will be a valuable resource for planarian research. These antibodies have the potential to be used to better understand planarian biology and to characterize phenotypes following RNAi experiments. In addition, we present alterations to fixation protocols and demonstrate how these changes can increase the labeling efficiencies of antibodies used to stain whole planarians.